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**Voltammetric Measurement of Oxygen in Single Neurons Using  
Platinized Carbon Ring Electrodes**

by

**Yau Y. Lau, Takayuki Abe, Andrew G. Ewing**

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**Department of Chemistry  
Penn State University  
University Park, PA 16802**



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VOLTAMMETRIC MEASUREMENT OF OXYGEN IN SINGLE NEURONS USING  
PLATINIZED CARBON RING ELECTRODES

Yau Yi Lau, Takayuki Abe and Andrew G. Ewing\*

Department of Chemistry  
152 Davey Laboratory  
Penn State University  
University Park, PA 16802

\* To whom correspondence should be addressed.

### ABSTRACT

Nafion-coated ultrasmall platinum ring electrodes have been implanted in the giant dopamine neuron of the pond snail, *Planorbis corneus* and the oxygen concentration inside these single neurons has been estimated. Experimental data suggest that the intracellular oxygen level in the identified dopamine neuron of *Planorbis corneus* is approximately 0.032 mM. The oxygen concentration immediately outside the cell (approx. 10  $\mu$ m away from the cell) is 0.041 mM. Furthermore, staircase voltammetry can be used to monitor dynamic changes in oxygen concentration inside the cell after bathing with Ringer's solution saturated with air/oxygen. Data obtained for intracellular oxygen concentrations suggest that intracellular oxygen consumption is increased following potassium chloride-induced stimulation of these cells.



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## INTRODUCTION

A key function of neurons is the generation, processing and transmission of impulses. In order to maintain the active potential and the normal function of neurons, a constant energy supply is required. Previous studies have shown that 40-50% of the total adenosine triphosphate (ATP) produced in nervous tissue is used for neuronal activities<sup>1-7</sup>. Glucose metabolism is the major energy source for the brain and is proportional to *in vivo* oxygen levels. In addition, oxygen is also closely linked to the synthesis, metabolism, release and uptake of neurotransmitters. Therefore, detection of oxygen in single neurons is a very important step toward understanding these cellular processes and the function of the brain at the single cell level.

Shibuki<sup>8</sup>, Erecinska *et. al.*<sup>9, 10</sup> and Fein *et. al.*<sup>11</sup> have shown that there is an increase in oxygen consumption following electrical stimulation of nerve tissue with a concomitant rise in the extracellular potassium concentration. This increase in oxygen consumption is  $\text{Ca}^{2+}$ -dependent and ouabain-resistant. Shibuki has also proposed that the increase in oxygen consumption represents the energy required for driving exocytotic release<sup>8</sup>.

Voltammetry is a very useful technique for measuring dissolved oxygen. Reviews of the electrochemistry<sup>12-14</sup> and the reduction mechanism of oxygen on different surfaces<sup>14-16</sup> can be found in the literature. The most commonly used oxygen probe is the Clark electrode, containing a platinum working electrode coated with electrolyte and covered with a gas permeable membrane such as polyethylene<sup>17</sup>. Recently, the successful fabrication of ultramicroelectrodes has allowed the real time measurement of electroactive substances in single cells<sup>18-23</sup>. Zimmerman and Wightman<sup>24</sup> have used fast-scan cyclic voltammetry in combination with Nafion-coated carbon disk electrodes for simultaneous measurement of oxygen and dopamine in the extracellular fluid of the rat brain. Uchida and coworkers<sup>25</sup> have determined the oxygen concentration in the cell *Bryopsis plumosa* using platinized silver ring electrodes and cyclic voltammetry. Dong and Kuwana

have also monitored oxygen and glucose during glucose oxidation using cobalt-porphyrin-Nafion films on carbon microarray electrodes and glucose oxidase on the outermost surface of the Nafion<sup>26</sup>. However, the direct monitoring of oxygen within a single neuron has not been reported to date.

In this paper, Nafion-coated ultrasmall platinized carbon ring electrodes have been used in conjunction with staircase voltammetry to determine oxygen levels in single cells of the pond snail, *Planorbis corneus*. In contrast to carbon surfaces, the platinum surface is a good catalyst for reduction of oxygen to water minimizing the formation of hydrogen peroxide which is toxic to the cell.

### EXPERIMENTAL

**Reagents.** Dopamine was purchased from Sigma Chemical Co., 5% Nafion from Solution Technology Inc. (Mendenhall, PA), ethanol from Midwest Grain Products Co. (Weston, MO), and oxygen and nitrogen from Union Carbide (Danbury, CT). All chemicals were used as received and all solutions were made with doubly distilled water (Corning Mega-Pure MP-3A). *In vitro* work was performed in a pH 7.4 snail Ringer's solution<sup>27</sup> (39 mM NaCl, 1.3 mM KCl, 4.5 mM CaCl<sub>2</sub>, 1.5 mM MgCl<sub>2</sub>, 6.9 mM NaHCO<sub>3</sub>) or in a pH 7.4 citrate/phosphate buffer. All solutions were purged with nitrogen for 20 min prior to carrying out experiments and a blanket of nitrogen was kept over the solutions at all times.

**Electrode Construction.** Ultrasmall carbon ring electrodes were constructed and voltammetrically tested as described previously<sup>18, 20, 21, 28</sup>. Carbon ring electrodes were initially tested in 1.0x10<sup>-4</sup> M dopamine in pH 7.4 citrate/phosphate buffer. Only electrodes which provided well-defined sigmoidal voltammograms with capacitive charging current below 10 pA were used. These electrodes were coated with platinum (1.0x10<sup>-3</sup> M H<sub>2</sub>PtCl<sub>6</sub> in 0.5 M H<sub>2</sub>SO<sub>4</sub>) by reduction at -0.10 V vs a sodium-saturated calomel reference electrode (SSCE) and characterized in 0.50 M H<sub>2</sub>SO<sub>4</sub> as

described elsewhere<sup>29</sup>.

A 0.5% Nafion solution was prepared by diluting a commercial solution (5% by weight in 80% lower aliphatic alcohols and 20% water) 10 times using 20% water and 80% ethanol. The electrodes were then dip-coated for 2 s and allowed to dry at room temperature overnight. Since electrodes vary in tip diameter from approximately 2 to 10  $\mu\text{m}$ , each electrode used was calibrated in standard solutions prior to and after *in vivo* measurements.

**Apparatus.** Cyclic and staircase voltammetry (-0.2 to 0.8, 50 steps, 20 mV/step and 100 ms/step) were carried out with an EI-400 potentiostat (Ensmann Instrumentation, Bloomington, IN). Potential waveforms for staircase voltammetry and data acquisition were generated with an IBM personal computer and a commercial interface (Labmaster, Scientific Solutions, Inc., Solon, OH). Electrochemical experiments employed a cell consisting of a 30 mL glass vial, filled to 25 mL, with holes drilled in a plastic cap to facilitate a three-electrode system. A platinum wire was used as the auxiliary electrode. All experiments were carried out in a copper mesh Faraday cage at room temperature.

***In vivo* Measurements and Local Bathings.** Intracellular voltammetry and local bathing were carried out as described previously<sup>21, 23</sup>. Briefly, *Planorbis corneus* were obtained from NASCO (Fort Atkinson, WI) and were maintained in aquaria at room temperature until used. The snails were pinned in a wax-filled petri dish and dissected under snail Ringer's solution to reveal the left and right pedal ganglia. A micromanipulator (Mertzhauser, Carl Zeiss, Inc., Germany) was used to place an electrode into the identified dopamine neuron<sup>30</sup>. The electrode potential was monitored with an oscilloscope vs a platinum wire during implantation. A negative shift in potential was indicative of cell penetration. Following implantation, the platinum ring working electrode was connected to the potentiost-

at. The SSCE reference electrode was placed via a salt bridge into the Ringer's solution with a platinum wire serving as the auxiliary electrode. All errors are reported as standard errors of the mean (SEM).

## RESULTS AND DISCUSSION

**Reduction of Oxygen.** Nafion-coated platinized carbon ring electrodes have been chosen for the present study instead of carbon ring electrodes for the following reasons. First, reduction of oxygen at carbon electrodes proceeds via an overall two-electron step to form hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) which is toxic to the cell. Second, slow kinetics for the reduction of oxygen result in less reversible voltammetric behavior at carbon electrodes, hence reduction of oxygen at carbon surfaces takes place at higher negative potentials (-1.2 V vs SSCE).

Figure 1 (a) shows a cyclic voltammogram for the reduction of oxygen in air-saturated Ringer's solution at a 2  $\mu\text{m}$  total tip diameter carbon ring electrode. The voltammogram for oxygen reduction is less Nerstian and does not achieve a limiting plateau in the potential range used (+0.8 to -1.2 V) indicating slow kinetics for oxygen reduction at carbon surfaces. In contrast, platinum surfaces are good catalysts for the reduction of oxygen to water with enhanced current due to a more efficient four-electron process. Despite these advantages platinum surfaces are generally considered to be more sensitive than carbon electrodes to changes in surface condition. Hence, a thin Nafion film has been coated on the platinum electrodes used here to reduce fouling after implantation inside cells.

Figures 1 (b) and (c) show cyclic voltammograms obtained in air saturated Ringer's solution for the reduction of oxygen at a 2  $\mu\text{m}$  tip diameter platinum ring electrode before and after Nafion coating. Although an expected reduction in the limiting current is observed at the Nafion-coated platinum ring electrode, this decrease is small. Oxygen has a high diffusion coefficient ( $D = 2 \times 10^{-6} \text{ cm}^2/\text{s}$ ) in Nafion<sup>31</sup> and readily permeates the film on the



electrode surface. The  $E_{1/2}$  for oxygen reduction is -120 mV and -220 mV for platinum and Nafion-coated platinum electrodes, respectively. This change in  $E_{1/2}$  is also reported by Lawson et. al.<sup>31</sup>.

Voltammograms of oxygen have been obtained in four standard solutions: nitrogen purged, air saturated, 50/50 nitrogen/oxygen purged, and an oxygen saturated Ringer's solution to establish calibration plots. Figure 2 shows the calibration plots for a platinum electrode before and after Nafion coating. The calibration plots are linear with correlation coefficients of 0.9997 and 0.9980 and slopes of 0.16 and 0.072 nA/mM for platinum and Nafion coated platinum electrodes, respectively.

**In Vivo Detection of Oxygen.** Figure 3 shows two representative voltammograms obtained when an electrode is placed approximately 0.4 cm and 10  $\mu$ m from the giant dopamine neuron of the pond snail, *Planorbis corneus*. Assuming oxygen is the only easily reduced species present at significant levels, the concentration of oxygen outside the neuron can be estimated from the *in vitro* calibration plots and the limiting current from the *in vivo* voltammograms. These results suggest that oxygen concentration is significantly lower near the neuron. At a distance 0.4 cm from the neuron the oxygen concentration is  $0.16 \pm 0.046$  mM ( $n = 3$ ), whereas the concentration 10  $\mu$ m from the cell is  $0.041 \pm 0.001$  mM ( $n = 3$ ). This trend has been observed with individual electrodes manipulated towards and away from the cell indicating that the change in limiting current is not due to electrode fouling. This last concentration is in surprisingly good agreement with the concentration range reported for oxygen in the extracellular fluid of the rat brain (25-40  $\mu$ M)<sup>24</sup>. In order to establish that the decrease in oxygen concentration observed as the electrode approaches the neuron is not due to a limiting of oxygen diffusion to the electrode, the following control experiments have been carried out. Voltammograms have been obtained with an electrode placed first 0.4 cm and then 10  $\mu$ m

from the bottom of the petri dish. As the electrode approaches the bottom of the petri dish there is no change in the oxygen voltammogram showing that a solid obstruction (petri dish or tissue) is not limiting oxygen diffusion to the electrode. These results strongly suggest that the decrease in oxygen concentration observed at locations in close proximity to the cell is the result of biological consumption of oxygen by the cell and the surrounding tissue.

Electrodes have been implanted in giant dopamine neurons of *Planorbis corneus* and voltammograms have been obtained. In all cases, the electrodes have been calibrated both before and after the cellular experiments. Figure 4 shows voltammograms obtained inside the cell with both a Nafion-coated platinum electrode and a carbon ring electrode. A reduction wave for oxygen is clearly observed when the platinum electrode is used. However, no reduction wave is apparent when the carbon ring electrode is used over the potential range from 0.3 to -0.6 V. This suggests that the reduction is due to oxygen at the platinum-coated electrode, since no wave is observed at the carbon ring electrode. It should be noted that other easily reduced species could also have poor electron transfer kinetics at carbon electrodes relative to platinum and this would result in the same effect observed here. The concentration of oxygen inside the cell has been estimated from the limiting current of voltammograms obtained inside several dopamine cells. This concentration is  $0.032 \pm 0.004$  mM (SEM,  $n = 13$ ). Air saturated Ringer's solution has an oxygen concentration of 0.24 mM. This suggests that oxygen transported into the cell is consumed immediately, presumably to provide energy for respiration and metabolism.

**Oxygen Transport into the Cell.** In order to evaluate the dynamics of oxygen transport into the cell, intracellular voltammetry has been used to monitor oxygen levels following application of solution baths to the cell. "Oxygen-free" (nitrogen purged), air-saturated, oxygen-saturated Ringer's

solution and air-saturated 3 M KCl in modified Ringer's solution (without  $\text{NaHCO}_3$ ) have been employed. Figure 5 shows a representative change in oxygen level following bathing with the various solutions. The average increase in oxygen concentration after bathing with "oxygen-free" solution, air-saturated solution, oxygen-saturated solution and 3 M KCl are  $0.010 \pm 0.0018$  mM (SEM,  $n = 3$ ),  $0.059 \pm 0.015$  mM (SEM,  $n = 3$ ),  $0.16 \pm 0.020$  mM (SEM,  $n = 3$ ) and  $0.029 \pm 0.003$  mM (SEM,  $n = 3$ ), respectively. The increase in signal after bathing is apparently due to the transport of oxygen into the cell. This transport is immediate and can be monitored by staircase voltammetry on these time scales. Oxygen levels then decrease back to baseline after approximately 2 min. In a simple-minded hypothesis, we attribute this decrease to the use of oxygen for cell respiration and metabolism. As would be expected, the largest increase in oxygen level is observed following application of oxygen-saturated Ringer's solution. A small increase in oxygen concentration after bathing with "oxygen-free" Ringer's solution might be attributed to the disturbance of the solution around the cell, although this is unclear.

If the transient changes in intracellular oxygen are solely related to the oxygen level in the bathing solution, then the increase in intracellular oxygen level is expected to be similar following bathing with either air-saturated 3 M KCl solution or air-saturated Ringer's solution. In vitro voltammetry in these two solutions provides similar results (Figure 6). However, the intracellular increase in oxygen level following application of air-saturated 3 M KCl in Figure 5 is significantly less than that observed after application of air-saturated Ringer's solution. We have modelled this difference by assuming that a large increase in intracellular oxygen consumption takes place following cell stimulation by high  $\text{K}^+$ . Several studies have suggested that a rise in extracellular potassium concentration, or electrical stimulation, results in an augmentation of cellular oxygen consumption<sup>8-10</sup>. One possibility is that an increase in

extracellular potassium concentration will stimulate exocytotic release of dopamine from the neuronal cell body. The stimulated release process would then lead to an increase in cellular oxygen consumption resulting from an increase in energy use for the synthesis of DA, vesicle transport, and exocytosis. This is also supported by Shibuki<sup>8</sup> where it has been suggested that the increase in oxygen consumption after cell stimulation represents the energy required for driving exocytotic release.

### CONCLUSIONS

The experimental results in this paper show that voltammetry at platinum electrodes is a useful technique for measuring oxygen concentrations in single neurons. The concentration of oxygen inside the giant dopamine cell of *Planorbis corneus* is 0.032 mM and the concentration 10  $\mu$ m from the outside of the cell is 0.041 mM under normal resting conditions for a dissected, living snail. The results also show that staircase voltammetry can be used to monitor the dynamics of oxygen transport from outside the plasma membrane to the inside. The average increases in oxygen concentration after bathing with "oxygen-free" solution, air-saturated solution, oxygen-saturated solution and 3 M KCl are  $0.010 \pm 0.0018$  mM (SEM, n = 3),  $0.059 \pm 0.015$  mM (SEM, n = 3),  $0.16 \pm 0.020$  mM (SEM, n = 3) and  $0.029 \pm 0.003$  mM (SEM, n = 3), respectively.

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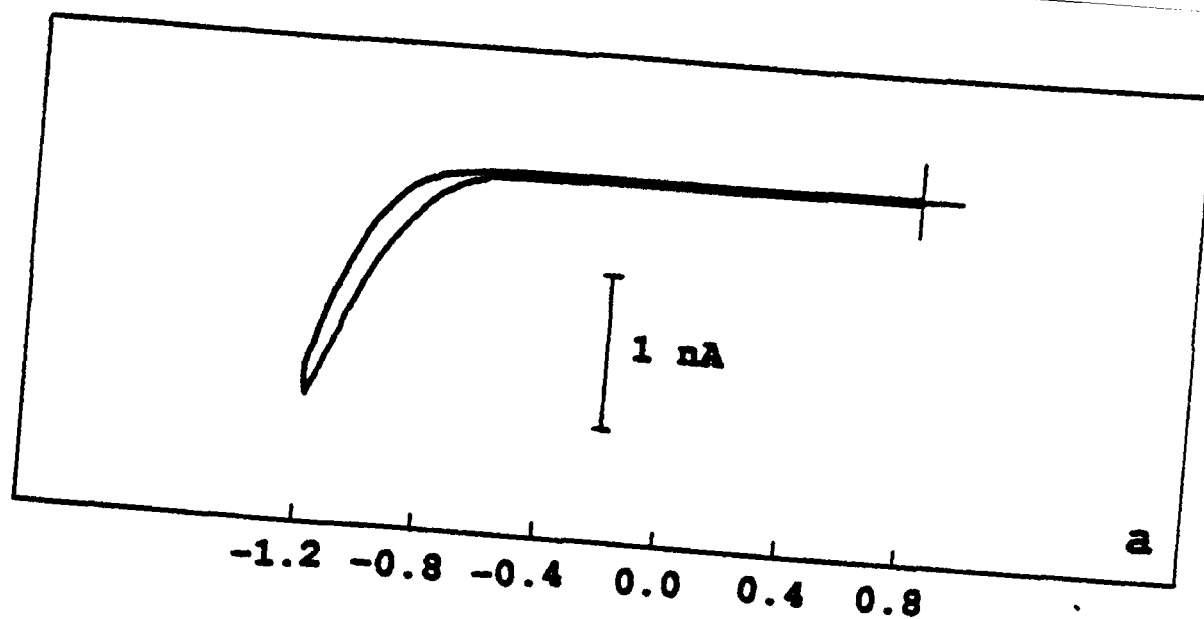
#### **UPCOMING RESEARCH**

Oxygen concentration inside single neurons has been measured using staircase voltammetry at Nafion-coated platinum ring electrodes.

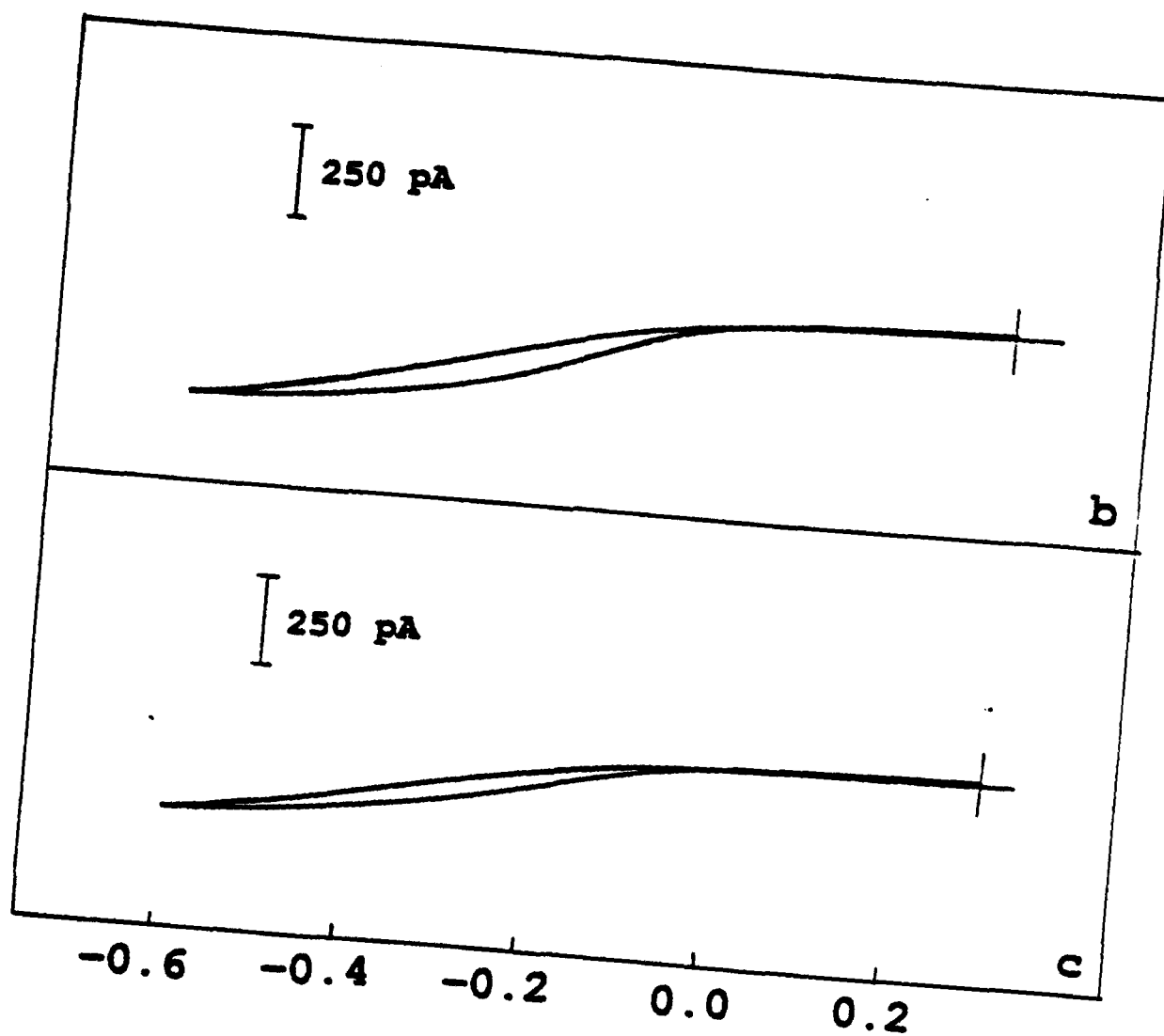


#### FIGURE CAPTIONS

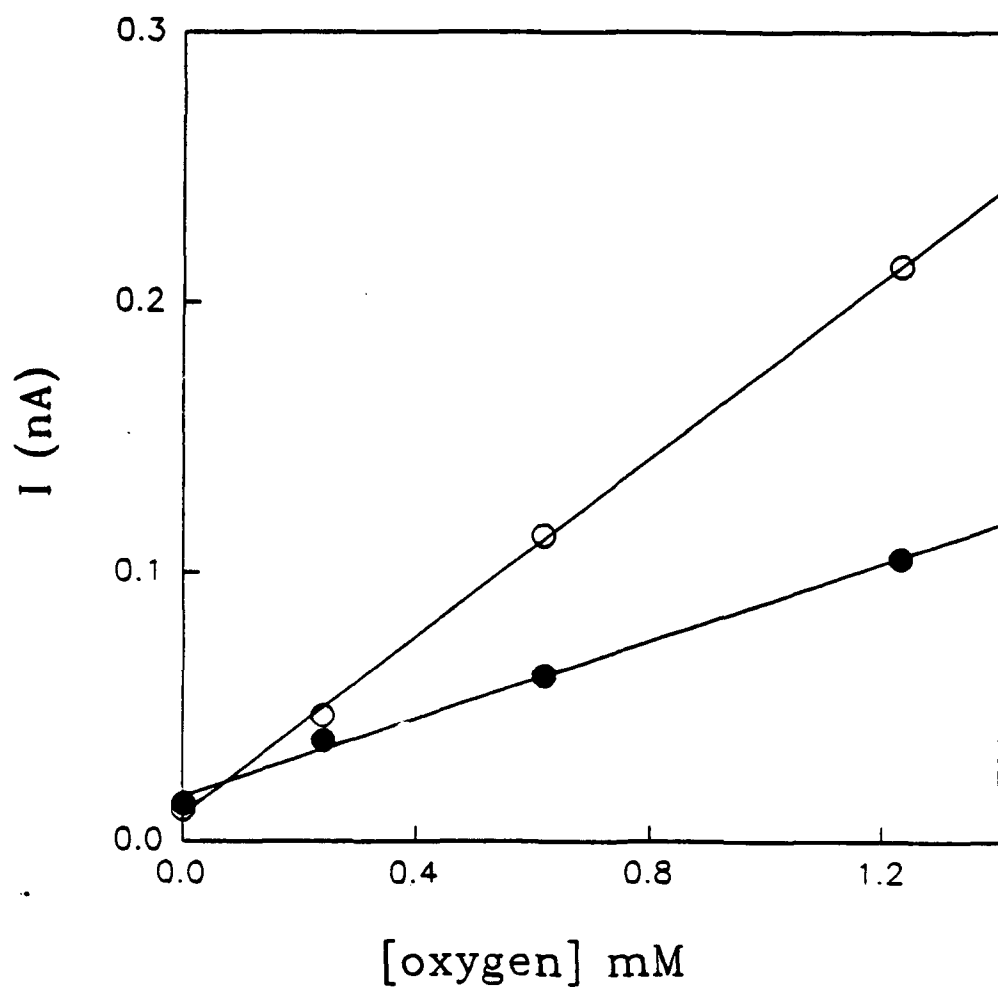
- Figure 1.** Cyclic voltammograms at the same electrode obtained at three stages of construction in air-saturated Ringer's solution. (a) Carbon ring electrode, (b) platinized carbon ring electrode (limiting current = 230 pA), (c) Nafion-coated platinized carbon ring electrode (limiting current = 170 pA). Scan rate = 200 mV/s.
- Figure 2.** Calibration plots for oxygen reduction at another 2- $\mu$ m tip diameter platinized carbon ring electrode before (open circles) and after (solid circles) coating with Nafion. Oxygen levels were varied from nitrogen purged to oxygen saturated. Scan rate = 200 mV/s.
- Figure 3.** Staircase voltammograms of oxygen obtained outside the large dopamine neuron of *Planorbis corneus*. A Nafion-coated platinized carbon ring electrode (2- $\mu$ m tip diameter) was placed (a) 10  $\mu$ m and (b) 400  $\mu$ m from the cell surface. Scan rate = 200 mV/s.
- Figure 4.** Staircase voltammograms obtained inside the giant dopamine cell of *Planorbis corneus* with a (a) 2  $\mu$ m Nafion coated platinum ring electrode and (b) 4  $\mu$ m carbon ring electrode (this lack of response was typical of all carbon ring electrodes in this potential range). Scan rate = 200 mV/s.
- Figure 5.** Plot of sampled current for repeated voltammograms in the cytoplasm of the large dopamine cell of *Planorbis corneus*. Arrows indicate times of bathing the cell with the following Ringer's solutions (in order): oxygen saturated; air-saturated; nitrogen-purged; 3 M KCl in air-saturated. Bathing volume = 50  $\mu$ L; scan rate = 500 mV/s; current measured at -0.6 V vs SSCE is plotted.
- Figure 6.** Reduction of oxygen at a 10- $\mu$ m platinized carbon ring electrode in air saturated Ringer's solution with (a) 3 M KCl added and (b) no extra KCl. Scan rate = 100 mV/s.

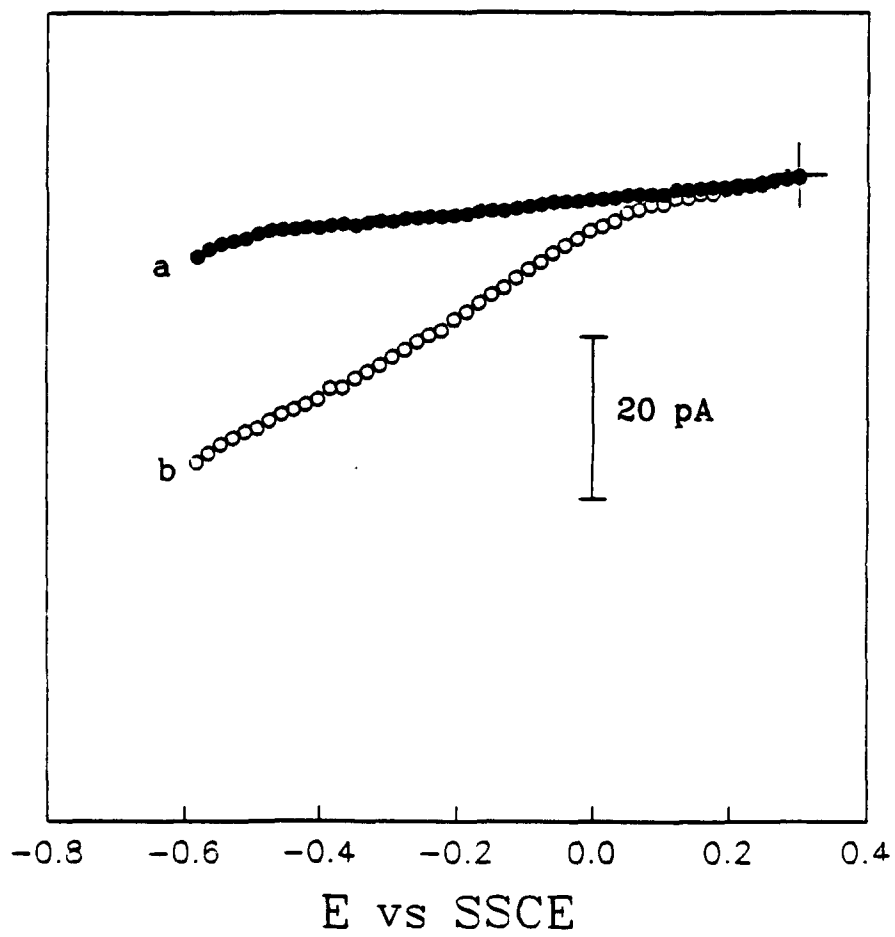


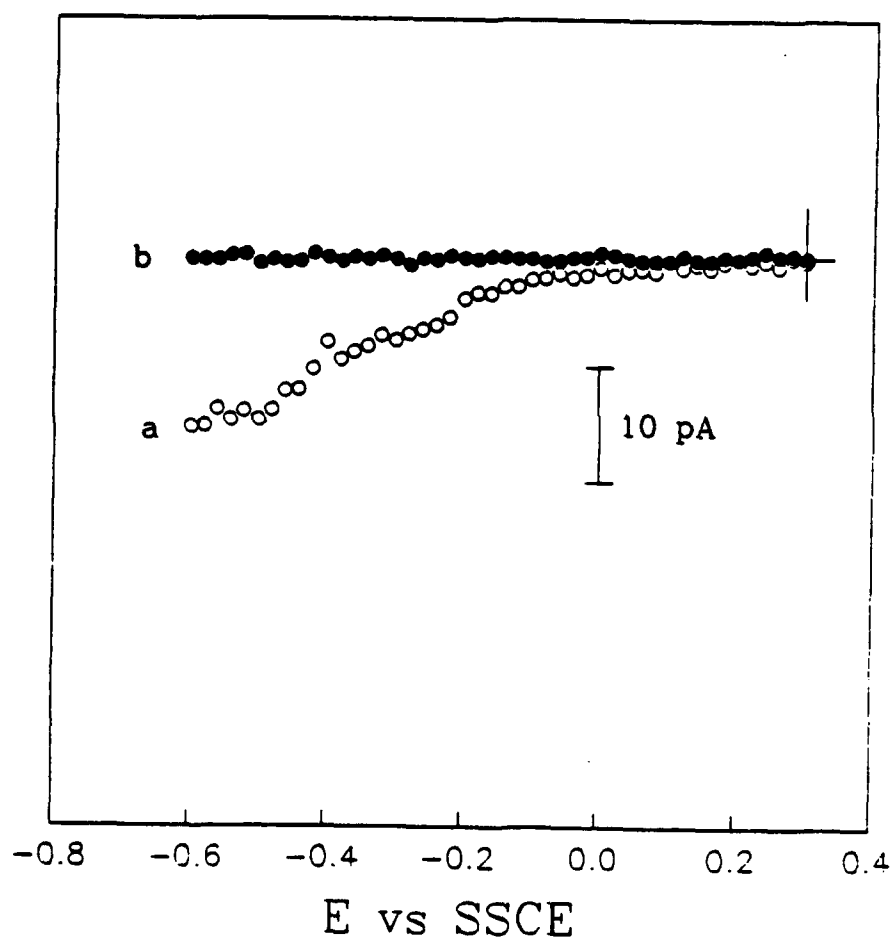
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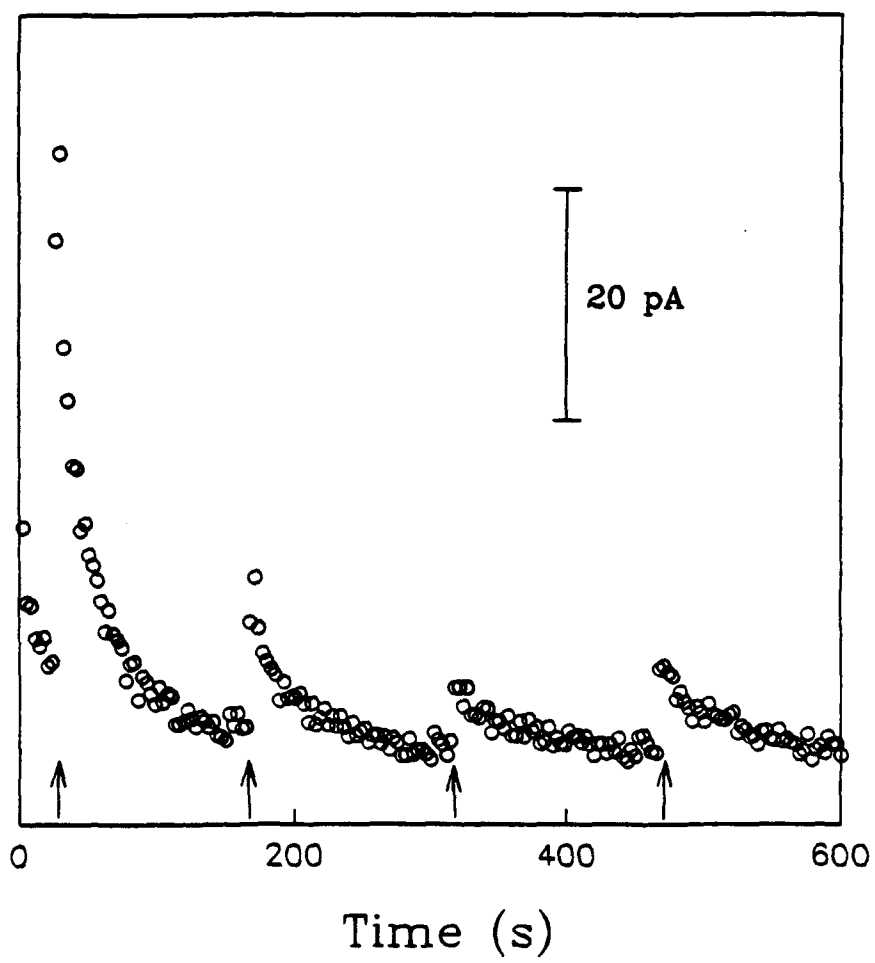


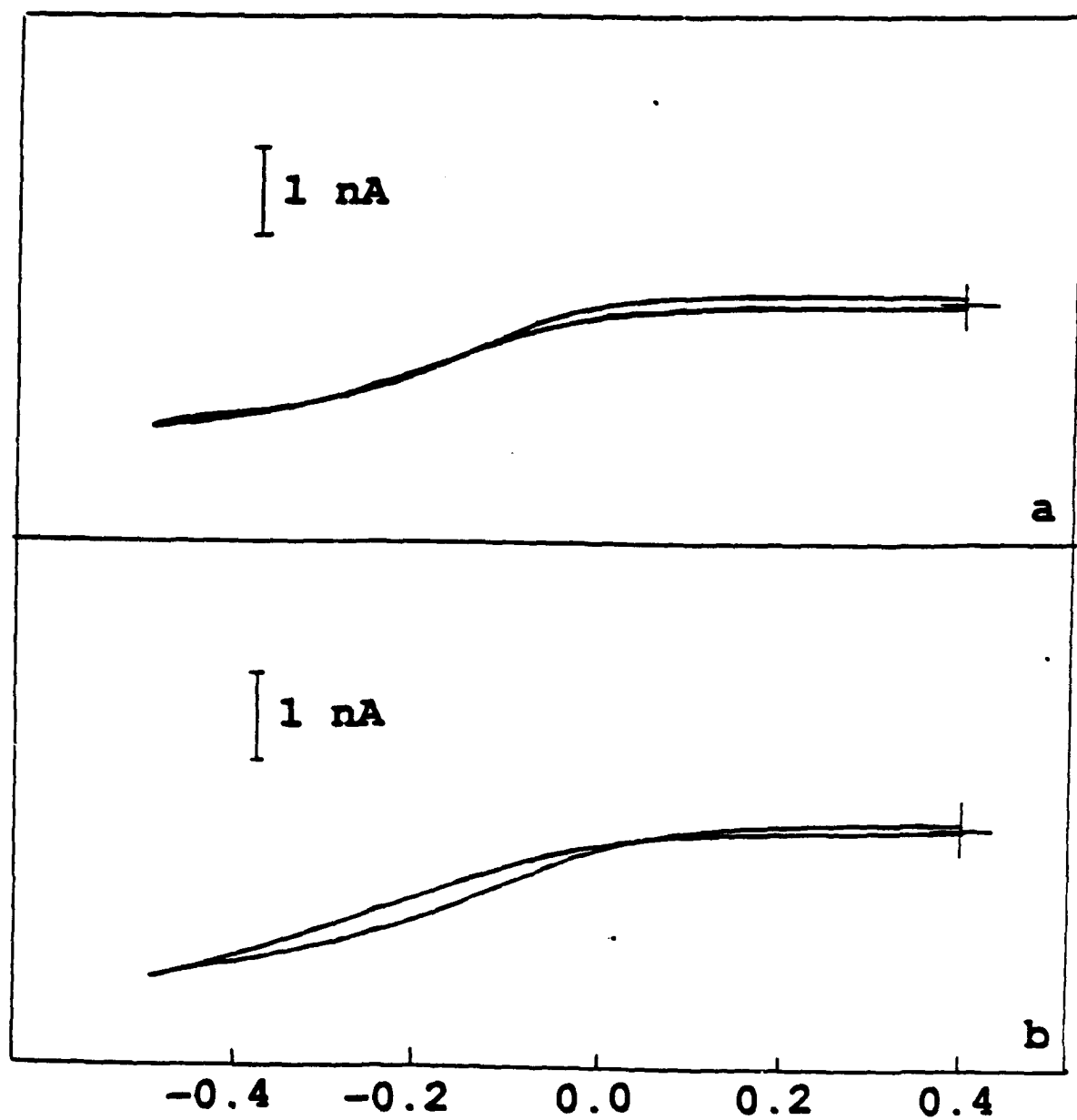
**E vs SSCE**











**E vs SSCE**